

B 13 size of INGAP monomer and reacts with antibody to INGAP in a Western analysis. The protein shares with INGAP peptide the ability to induce ductal cell proliferation.

Remarks

Amendments to the claims are shown on the attached sheets at the end of this paper. The status of the claims and the support for the claim changes are also shown on the attached sheets.

Surrender of Patent Grant

Applicants submit herewith and surrender the patent grant on the condition of grant of a reissue patent.

Claim Amendments Filed July 19, 2001.

New claims 48 and 49 and the amendments to claims 7, 8 and 15 are resubmitted to comply with 37 C.F.R. 1.121(b) and 37 C.F.R. 1.173.

Oath/Declaration

The oath/declaration has been objected to for containing non-initialed and/or non-dated alterations. A supplemental declaration executed by Dr. Ronit Rafaeloff-Phail will be submitted to comply with 37 CFR §1.52(c).

Claim Amendments

Please amend claims 1, 7, 8, 10, 13-18, 23, 38, 45-46, and 48-49 as follows.

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1. (Amended) A recombinant construct for expression of Islet Neogenesis Associated Protein or INGAP activity comprising:

a first nucleotide sequence encoding amino acid[s] residues 27 to 175 as shown in SEQ ID NO: 6 operably linked to a transcriptional initiation site and a translational initiation site, wherein a second nucleotide sequence encoding a signal peptide is not present immediately 5' of said first nucleotide sequence.

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7. (Amended) The construct of claim 1 [further comprising a promoter sequence] wherein the transcriptional initiation site is capable of initiating constitutive transcription.

8. (Amended) The construct of claim 7 wherein the [promoter sequence] transcriptional initiation site is Rous sarcoma virus long terminal repeat (RSVLTR).

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10. (Amended) The construct of claim 9 wherein the nuclear antigen is Epstein-Barr nuclear antigen-1 (EBNA-1).

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13. (Amended) A method of producing biologically active Islet Neogenesis Associated Protein or INGAP [protein] from a recombinant host cell comprising the steps of:

culturing a host cell comprising a recombinant construct comprising a first nucleotide sequence encoding amino acid[s] residues 27 to 175 as shown in SEQ ID NO: 6 operably linked to a transcriptional initiation site and a translational initiation site, wherein a second nucleotide sequence encoding a signal peptide is not present immediately 5' of said first nucleotide sequence, and

recovering protein from said cultured host cell.

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continue
14. (Amended) The method of claim 13 wherein the construct further comprises a third nucleotide sequence encoding a histidine tag, and INGAP [protein] is purified using a nickel affinity matrix.

15. (Twice Amended) A host cell comprising a recombinant construct comprising a first nucleotide sequence encoding amino acid[s] residues 27 to 175 as shown in SEQ ID NO: 6 operably linked to a transcriptional [iron] initiation site and a translational initiation site, wherein a second nucleotide sequence encoding a signal peptide is not present immediately [5+] 5' of said first nucleotide sequence.

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16. (Amended) The construct of claim 1 wherein the first nucleotide sequence encoding amino acid[s] residues 27 to 175 comprises nucleotides 12-456 of SEQ ID NO: 4.

17. (Amended) The method of claim 13 wherein the first nucleotide sequence encoding amino acid[s] residues 27-175 comprises nucleotides 12-456 of SEQ ID NO: 4.

18. (Amended) the host cell of claim 15 wherein the first nucleotide sequence encoding amino acid[s] residues 27-175 comprises nucleotides 12-456 of SEQ ID NO: 4.

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21. (Amended) A pair of oligonucleotide primers for amplifying a portion of the human INGAP coding sequence consisting of nucleotides 12 to 456 of SEQ ID NO: 4, wherein said portion excludes the nucleotides encoding the signal peptide, wherein each of said oligonucleotide primers hybridizes to an opposite strand of a double-stranded INGAP template, wherein a first of said oligonucleotide primers hybridizes to the 5' end of the coding sequence for mature human INGAP and the second of said oligonucleotide primers hybridizes to the 3' end of the nucleotide sequence encoding mature human INGAP.

23. (Amended) A method of making an expression construct for producing INGAP in a recombinant host cell, comprising the step of:

B20 linking a transcription initiation site, a translation initiation site, and a coding sequence for mature human INGAP consisting of nucleotides 12 to 456 of SEQ ID NO: 4, to make an expression construct which is devoid of the signal sequence of the coding sequence of INGAP.

Sub C4
B21 27. (Amended) The method of claim 23 wherein the coding sequence for mature human INGAP is obtained by amplification of a portion of the human INGAP coding sequence consisting of nucleotides 12 to 456 of SEQ ID NO: 4, wherein said portion excludes the nucleotides encoding the signal peptide.

B22 29. (Amended) A recombinant construct for expression of Islet Neogenesis Associated Protein (INGAP) activity comprising:

DC5 a first nucleotide sequence encoding mature human INGAP consisting of nucleotides 12 to 456 of SEQ ID NO: 4, said first nucleotide sequence being operably linked to a transcriptional initiation site and a translational initiation site, wherein a second nucleotide sequence encoding a signal peptide according to SEQ ID NO: 5 is not present immediately 5' of said first nucleotide sequence.

B23 38. (Amended) The construct of claim 37 wherein the nuclear antigen is Epstein-Barr nuclear antigen-1 (EBNA-1).

B24 45. (Amended) A method of producing biologically active Islet Neogenesis Associated Protein (INGAP) from a recombinant host cell comprising the steps of:

culturing a host cell comprising a recombinant construct comprising a first nucleotide sequence encoding mature human INGAP consisting of nucleotides 12 to 456 of SEQ ID NO: 4 operably linked to a transcriptional initiation site and a translational initiation site, wherein a

second nucleotide sequence encoding a signal peptide according to SEQ ID NO: 5 is not present immediately 5' of said first nucleotide sequence; and

recovering protein from said cultured host cell.

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Conclude
46. (Amended) The method of claim 45 wherein the construct further comprises a third nucleotide sequence encoding a histidine tag, and INGAP is purified using a nickel affinity matrix.

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48. (Amended) The method of claim 23 wherein the coding sequence for mature human INGAP encodes amino acid residues 27 to 175 as shown in SEQ ID NO: 6.

49. (Amended) The pair of oligonucleotide primers of claim 21 wherein the first of said oligonucleotide primers comprises nucleotides 12 to 31 of SEQ ID NO: 2 and the second of said oligonucleotide primers comprises nucleotides 13 to 32 of SEQ ID NO: 3.